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Modeling chronic psoriatic inflammation in a 3D reconstructed skin

I Lorthois[1] and R Pouliot[2] *1 Centre LOEX de l'Université Laval, Génie Tissulaire et Régénération, Centre de Recherche FRQS du CHU de Québec, Axe Médecine Régénératrice, Quebec, Canada and 2 Faculté de Pharmacie, Université Laval, Quebec, Canada*

Macrophages and Th17 lymphocytes play a key role in inducing psoriasis-like skin disease. However, the specific role of each component for the activation of epithelial cells remains to be clarified. Here, we aimed to define the specific involvement of macrophages and lymphocytes in a 3D reconstructed skin model to better understand the complex link between psoriatic epithelial cells and immune cells that underlies the typical inflammatory vicious circle of psoriasis. Monocyte-derived macrophages and T cells were isolated from human blood and seeded on the dermal compartment of healthy or psoriatic tissues reconstructed according to the self-assembly approach. Immunolabeling analyses of involucrin, filaggrin, loricrin, transglutaminase were performed to identify which cells were responsible for the abnormal differentiation of keratinocytes. The presence and the migration of leucocytes through skin substitutes were examined thanks to anti-CD163 and CD3 stainings. The expression level of specific cytokines and chemokines, such as MCP-1, IL-6, and IL-1β, were further assessed by ELISA. Our results showed that both macrophages and T cells were homogeneously dispersed throughout the dermis. Macrophages incorporated into healthy substitutes appeared to modify the expression of early epidermal differentiation markers toward an inflammatory phenotype, such as observed with psoriatic cells. T cells affected early and late keratinocyte differentiation markers toward a psoriatic phenotype. Moreover, expression levels of pro-inflammatory cytokines increased in healthy immunocompetent substitutes over the air-liquid culture time frame compared to immunodeficient models. Both innate and adaptive immune cells contribute to keratinocyte deregulation and these results strongly suggest that this unique immunocompetent model would be useful in the discovery of new therapeutic targets for the treatment of inflammatory chronic skin diseases, such as psoriasis.

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Lupus Ro60 autoantigen cross-reactivity with commensal Ro60 orthologs

T Greiling[1], C Dehner[2], X Chen[2], K Hughes[2], S Vieira[2], W Ruff[2], S Sim[2], S Wolin[2] and M Kriegel[2] *1 Oregon Health and Science University, Portland, OR and 2 Yale Unviersity, New Haven, CT*

Anti-Ro60 autoantibodies are some of the earliest found in lupus patients and initiate epitope spreading. We identified a subset of commensal microbes with Ro60 orthologs, and hypothesized that Ro60 cross-reactivity may initiate or flare lupus in genetically susceptible individuals. Subjects with systemic and subacute cutaneous lupus erythematosus and healthy controls were recruited and 16S V4 sequencing of the skin, oral, and fecal microbiomes was performed. The presence of commensals with Ro60 orthologs was common among healthy and lupus subjects. Ro60+ lupus subjects had higher mean levels of *P. propionicum* on the skin than healthy subjects but there was no overall dysbiosis of the microbiota. Lupus memory T cell clones specific for *P. propionicum* proliferated in response to human Ro60. Similarly, T cell clones specific for *B. thetaiotaomicron* Ro60 proliferated in response to human Ro60, demonstrating T cell cross-reactivity. Human Ro60+ lupus serum immunoprecipitated *P. propionicum* Ro60 and its Y RNA binding partner, suggesting B cell cross-reactivity. Mice monocolonized with *B. thetaiotaomicron* produced serum anti-Ro60 antibodies, and cells from spleen and mesenteric lymph node proliferated in response to both *B. thetaiotaomicron* Ro60 and human Ro60, demonstrating cross-reactivity and the potential for causality. In summary, Ro60 autoimmune T and B cells from human lupus patients reacted with commensal Ro60 *in vitro*, and commensal Ro60 triggered anti-human Ro60 responses *in vivo*. Taken together, these data support that colonization with Ro60 ortholog-producing bacteria may induce and sustain chronic autoimmunity in lupus. This concept may apply more broadly to human autoimmune diseases and could lead to development of novel microbiota-targeted approaches to treat autoimmunity.

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Expression of αvβ8 by Langerhans cells is required for Th17 differentiation and tethering of LC in the epidermis

S Kashem[1], J Mohammed[1] and D Kaplan[2] *1 University of Minnesota, Minneapolis, MN and 2 University of Pittsburgh, Pittsburgh, PA*

Langerhans cells (LCs) reside in the epidermis where they capture cutaneous antigens and migrate to the skin draining lymph nodes (sLN) to prime and differentiate T cells. Using a murine skin infection model, our group has recently demonstrated that Langerhans cells were both necessary and sufficient to drive T helper 17 (Th17) differentiation in response to epicutaneous *Candida albicans* infection *in vivo*. Th17 differentiation requires a combination of IL-1β, IL-6, IL-23 and TGFβ. LC-derived IL-6 but not IL-1β, IL-23 or TGFβ was required for Th17 differentiation. Although LC-derived TGFβ was not required for Th17 differentiation, we found that activation of latent TGF-β by the integrin αvβ8 expressed by LC was required for efficient Th17 differentiation during *C. albicans* infection. Notably, the constitutive absence of TGFβ or αvβ8 on LC did not affect Treg numbers or phenotype. We have previously shown that activation of latent TGFβ by αvβ8 expressed by keratinocytes in $Itgb8^{\Delta KC}$ mice is required to inhibit spontaneous migration of LCs from the epidermis to sLN. LC migration under steady state and inflammatory conditions, however, was not affected in $Itgb8^{\Delta LC}$ mice. In contrast, tamoxifen induced ablation of αvβ8 in $Itgb8^{TAM\Delta LC}$ mice prevented LC migration. Over time, LC were observed in the epidermis near the stratum granulosum and were significantly reduced from both the epidermis and sLN. Thus, activation of TGFβ via αvβ8 expressed by LC is required for efficient Th17 differentiation in the setting of *C. albicans* infection. αvβ8 on LC is also required for appropriate epidermal localization of LC but this is likely compensated by other factors in the setting of a constitutive loss of this integrin.

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Liquid crystalline ordering of antimicrobial peptide-RNA complexes controls TLR3 activation

EY Lee[1], T Takahashi[2], T Curk[3], J Dobnikar[4], RL Gallo[2] and GCL Wong[1] *1 Department of Bioengineering, UCLA, Los Angeles, CA, 2 UCSD, San Diego, CA, 3 Department of Chemistry, University of Cambridge, Cambridge, United Kingdom and 4 International Center for Soft Matter Research, Beijing University of Chemical Technology, Beijing, China*

Double-stranded RNA (dsRNA) can induce potent production of pro-inflammatory cytokines in normal human epidermal keratinocytes (NHEK) by binding to endosomal Toll-like receptor-3 (TLR3). It is also known that the cationic human antimicrobial peptide LL37 forms electrostatic immune complexes with self-dsRNA to hyperactivate TLR3 in psoriatic keratinocytes. Here, we combine X-ray scattering experiments and computer simulations with measurements of NHEK cytokine production to elucidate a selection rule for cationic molecules that electrostatically condense dsRNA and activate TLR3. TLR3 activation intimately depends on the inter-RNA spacing and repeat number of parallel dsRNA molecules in the liquid-crystalline self-assembled complexes. Complexes that present dsRNA at the optimal spacing can engage multiple TLR3 receptors simultaneously, driving receptor clustering, super-selective receptor binding, and downstream immune amplification. We not only demonstrate the structural basis for LL37-mediated hyperactivation of NHEK in psoriasis, but also illustrate how to deterministically modulate immune responses in the skin by controlling the inter-RNA spacing within self-assembled polycation-dsRNA complexes.

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Evaluation of soluble IgE receptors, sCD23, sFcεRI and Galectin-3, in sera from patients with Bullous pemphigoid

H Holahan[1], J Fairley[2] and K Messingham[2] *1 Rutgers - NJMS, Newark, NJ and 2 University of Iowa, Iowa City, IA*

Bullous pemphigoid (BP) is one of many autoimmune diseases characterized by IgE autoantibodies. Understanding how IgE participates in the pathogenesis of BP is complex due to its interaction with both membrane bound and soluble receptors. The goal of the current study was to evaluate circulating levels of all three soluble IgE receptors, sCD23, sFcεRI and Galectin-3, in untreated BP patients and matched controls. Further, to better understand how these receptors might be involved in BP, soluble receptor levels were correlated with disease severity, circulating IgG and IgE autoantibody levels and peripheral eosinophil count. The study examined 39 patients with BP (24 females, 15 males, mean 78.2 years, range 59 - 97) and 38 healthy controls (21 female, 17 male, mean 78.4 years, range 64-98) with no history of autoimmunity or immunosuppressive therapy. Patients were scored for disease activity and serum autoantibody and receptor levels were measured by ELISA. As expected, most BP patients had significantly ($p< 0.001$) elevated circulating levels of BP180 IgG, BP230 IgG, BP180 IgE, total IgE, and eosinophils. Additionally, levels of all three soluble IgE receptors, sCD23, sFcεRI and Galectin-3, were significantly higher in patients with BP compared to controls ($p \leq 0.05$). A Spearman's analysis revealed that levels of sCD23 correlated with BP180 IgG ($r = 0.348$, $p \leq 0.05$) and circulating eosinophil count ($r = 0.485$, $p \leq 0.01$), but did not correlate with circulating or BP 180-specific IgE. Levels of sFcεRI correlated with BP 180 IgE ($r = 0.496$, $p \leq 0.01$) and Galectin-3 levels did not correlate with any of the parameters measured. The correlation of IgE with sFcεRI, but not sCD23 or Galectin-3, likely reflects differences in the regulation of receptor expression. However, the global elevation of these receptors in BP suggests that manipulation of soluble IgE receptors may provide an additional therapeutic avenue.

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Type I interferon signaling suppresses the development of vitiligo as well as the response to melanoma immunotherapy

RL Riding[1], K Fukuda[1], JM Richmond[1] and JE Harris[2] *1 University of Massachusetts Medical School, Worcester, MA and 2 Department of Dermatology, University of Massachusetts Medical School, Worcester, MA*

Type I interferons, are cytokines originally recognized for their anti-viral properties. However, type I interferons are pleiotropic, broadly modulating both innate and adaptive immune responses. For example, high levels of type I interferons are known to drive autoimmunity in diseases such as systemic lupus erythematosus, yet are beneficial as a therapy for patients with multiple sclerosis. Interestingly, case studies show that HCV patients given PEGylated type I interferon injections can lead to the development of vitiligo. Vitiligo is an autoimmune skin disease in which melanocytes are killed by autoreactive CD8 T cells specific for melanocyte/melanoma-shared antigens, such as premelanosome protein (PMEL). We developed a mouse model of vitiligo through adoptive cell transfer (ACT) using PMEL-specific CD8 T cells (PMELs) and found that vitiligo pathogenesis is primarily driven by IFNg. We then sought to determine the role of type I interferons in the development of vitiligo and discovered that interferon a-receptor deficient ($IFNAR^{-/-}$) hosts develop accelerated and more severe vitiligo compared to WT mice. We observed a similar result in a spontaneous model of vitiligo that was not dependent on ACT. Subsequent studies showed that IFNAR expression was not required on PMELs or other immune cells to suppress vitiligo, but rather on radioresistant cells. To determine whether this observation could be useful in melanoma immunotherapy, we tested ACT of PMELs in B16F10 melanoma-inoculated $IFNAR^{-/-}$ and WT mice and found that $IFNAR^{-/-}$ mice were relatively protected from the tumor, with a lower tumor burden and an elevated number of tumor-infiltrating PMELs compared to WT hosts. Collectively, these results strongly suggest that type I interferon signaling suppresses anti-melanocyte immune responses in both autoimmunity and tumor immunity.